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The Dynamics of Insulin Release from Monolayer-Cultured Pancreatic Cells in a New Perifusion System (39905)

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The monolayer culture of pancreatic cells has been widely employed for the study of morphology and function of A and B cells of fetal or adult pancreas from various animals and humans (1-8).

However, functional studies of hormonal secretion of monolayer cultures have been carried out by static incubation procedures, and the conclusions drawn from these experiments are limited, because the response of the perfused or intact pancreas is known to be dynamic.

In this report, we describe a perifusion system for cultured cells in monolayer, and report the dynamics of insulin release in this system.

Materials and Methods. (i) Preparation of monolayer cell culture. The method of monolayer culture of pancreas from neonatal rats reported by Lambert *et al.* (2) was used. Briefly, after pancreata of new born rats (4 to 5 days old) were excised and minced, the pancreatic tissue was treated in a mixture of trypsin (0.2%) and collagenase (0.01%) in phosphate-buffered saline (pH 7.7, Ca^{2+}, Mg^{2+} free) at 37°. Isolated cells were collected and resuspended in culture medium (TCM 199) containing 10% fetal calf serum, 300 mg/100 ml of glucose, and 400 IU/ml of penicillin G. All procedures were performed under sterile conditions.

Plastic petri-type tissue culture dishes (No. 3002, 60 × 15 mm, Falcon Plastics) with cell suspensions were kept in an incubator at 37°, under 5% CO_2 and 95% air. About 15 hr after starting the culture, the supernatants were decanted into new petri dishes to remove as many fibroblastoid cells as possible. Cell cultures were used for perifusion studies 3-4 days after decantation. Generally, cells from five newborn pancreata were used for one perifusion.

(ii) Perifusion system. The perifusion system consisted of (a) reservoirs, (b) a perifusion chamber, (c) a filter, (d) a peristaltic pump, and (e) a fraction collector, as illustrated in Fig. 1.

The reservoirs contained Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4), containing 0.5% dialyzed bovine serum albumin (Behringwerke AG), and were gassed continuously with a mixture of 95% O_2 and 5% CO_2 in a constant-temperature water bath at 37°.

The perifusion chamber, labeled "metal chamber" in Fig. 1, was composed of the cut-off bottoms of two petri dishes, on which the pancreatic cells were cultured, and a silicon-rubber square sheet (thickness, 2 mm) with a rhomboid hole in the center. The chamber was made by placing petri dishes on both sides of the sheet and fixing them in place with square stainless-steel plates with screws at each corner. This chamber was basically the same as that described previously (5). Two 18-gauge injection needles were introduced to this chamber by piercing sideways through the silicon-rubber sheet. Needles were placed at the corners of the rhomboid hole, as indicated with arrows in Fig. 1.

The perifusate was pumped into the chamber through these needles using a peristaltic pump (Perpex 10200, LKB Co.) and was collected in a fraction collector (Ultrarac 7000, LKB Co.) after filtration through a glass-fiber filter (No. AP 200100, Millipore Filter Corp.). The flow rate of perifusate was fixed at 0.8 ml/min, and samples were collected every 2 min. Homogeneous distribution of perifusate in the perifusion chamber at this flow rate was

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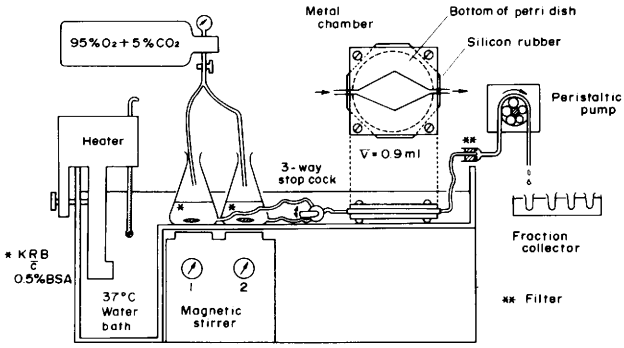

FIG. 1. Perifusion system for monolayer cultures of pancreatic cells. Top view and side view of the perifusion chamber are illustrated in the middle as "metal chamber." The rhomboid hole in the silicon-rubber sheet is drawn with solid lines at the center. The outer edge of the silicon-rubber sheet, shaped octagonally, is illustrated by solid and dashed lines. The edge of the bottom of the petri dish is drawn with broken lines. The square drawn with solid lines indicates the metal plate.

confirmed by examining with buffer containing a dye.

(iii) Assay method. The concentration of immunoreactive insulin (IRI) in the perifusate was determined by a double-antibody method (9), using rat insulin standards. Glucose was determined using a glucose oxidase method (10).

Results. As shown in Fig. 1, the net volume of the perifusion chamber was 0.9 ml (45 × 20 × 2 × 0.5 mm). This volume, plus any dead space, was replaced within 8–10 min when the perifusate was changed suddenly using a three-way stopcock and a flow rate of 0.8 ml/min. As shown in Fig. 2, the change in glucose concentration from 50 to 100 or to 300 mg/100 ml was reasonably rectangular.

Figure 2 shows the effect of glucose on insulin release in this perifusion system. The insulin release gradually declined during the exposure to glucose at 50 mg/100 ml, and became stable after 30 to 40 min, remaining constant for the following 2 hr.

When the glucose concentration was raised to 100 or 300 mg/100 ml, the release of insulin increased, demonstrating a prominent initial peak followed by a second phase, which lasted until the cessation of stimulation. In preliminary experiments, we obtained a reproducible glucose effect on insulin release for at least 120 min. Therefore, all subsequent perifusion experiments were carried out for 120 min or less.

The pattern of insulin release which was found with glucose alone (300 mg/100 ml)

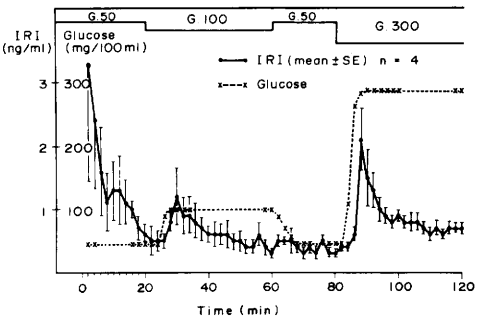

FIG. 2. Effect of glucose on insulin release by perifused cell cultures from neonatal rat pancreas. Insulin release was expressed by the concentration (nanograms per milliliter). The output of insulin is the "indicated concentration" × 0.8 (nanograms per minute).

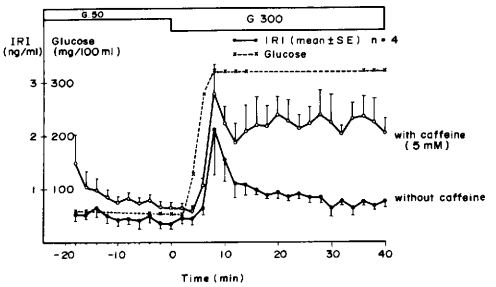

FIG. 3. Effect of caffeine (5 m*M*) on insulin release induced by glucose (300 mg/100 ml).

or with glucose and caffeine (5 m*M*), in the same preparation, is shown in Fig. 3. A biphasic increase of insulin release was clearly demonstrated at this concentration of glucose. Both phases were elevated by the addition of caffeine (5 m*M*). Indeed,

caffeine caused approximately a threefold increase in the second phase.

As shown in Fig. 4, tolbutamide (100 μg/ml) was a strong stimulator of insulin release at 50 mg/100 ml of glucose. A prominent first peak, followed by a significant second phase, was demonstrated.

As shown in Fig. 5, the stimulating effect of tolbutamide (100 μg/ml) decreased as the culture became older, although the pattern of insulin release was maintained. This change may have been due to the proliferation of fibroblasts and the relative decrease of endocrine cells in the 7-day culture, compared with the 4-day culture, observed by phase-contrast microscopy.

Discussion. Endocrine pancreas cultured in monolayer has been shown to release insulin in response to the usual stimuli using static incubation procedures (1, 2, 5), although the absolute amount of hormone released decreased day by day (1). These cultures are particulary convenient for studying the morphological changes of B and A cells in correlation with their function (6). To date, perifusion techniques have been employed only for the study of isolated islets or pieces of pancreas (11, 12). In this report, we have shown that the perifusion technique can be applied to the study of the endocrine function of pancreatic cells cultured in monolayer, and that these cells have a dynamic pattern of response similar to that of other systems.

However, the first peak of insulin release induced by glucose or other secretagogues was rather larger than the second phase, when compared to the pattern obtained with isolated islets or perfused pancreas, although a similar smaller secondary phase

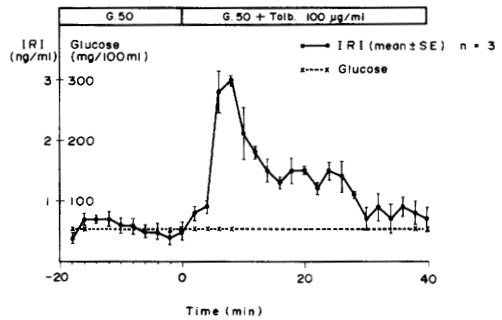

FIG. 4. Effect of tolbutamide (100 μg/ml) on insulin release with 50 mg/100 ml of glucose.

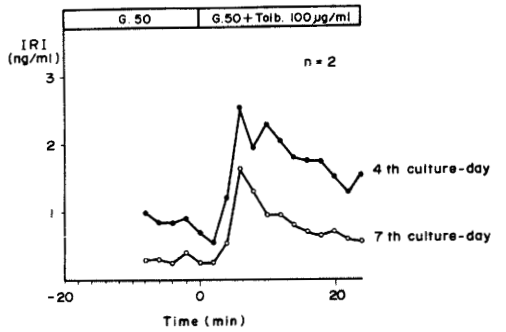

FIG. 5. Relationship between insulin release and age of the culture tested by tolbutamide (100 μg/ml). Points are means of two observations.

of insulin release has been observed in perifused pancreatic tissue from fetal or neonatal rats (13, 14). Thus, this pattern may be due to inmaturity of neonatal pancreas or to the conditions of monolayer culture.

The marked effect of caffeine (5 m*M*) was almost the same as that reported for pieces of fetal pancreas (13, 15). Clearly, in the future it will be necessary to compare the dynamics of insulin release from cultures of fetal and adult rat pancreata at various stages of maturity.

Using neonatal rat pancreas, we observed that tolbutamide (100 μg/ml) stimulated both phases of insulin secretion at a low concentration of glucose (50 mg/100 ml). This is somewhat different from the results of perifusions employing isolated islets (11) or fragments of pancreas of adult rat (16). This significant stimulation of the second phase of insulin release by tolbutamide may be due to the presence of glucose, as found by Grodsky *et al.* (17), using perfused adult rat pancreas in the presence of 100 mg/100 ml of glucose.

We conclude that the perifusion system of monolayer cell cultures described here is useful for studying the dynamics of insulin release induced by various stimuli. This system may also be applicable to the study of the dynamics of hormonal secretion from other endocrine tissues which can be cultured in monolayer (18–20).

Summary. A method for the perifusion of monolayer cultures of endocrine pancreas from neonatal rats is described. Isolated cells were perifused in a chamber composed of modified petri dishes used for the primary culture. Biphasic insulin release was ob-

served following stimulation with glucose and tolbutamide. Although the output declined after the fourth day of culture, the pattern of release remained similar, indicating the usefulness of this technique for dynamic studies of hormonal secretion.

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